LARVAL TREMATODES FROM AUSTRALIAN FRESHWATER MOLLUSCS, PART XI

By T. HARVEY JOHNSTON AND ANNE C. BECKWITH, UNIVERSITY OF ADELAIDE.

Fig. 1–18.

EARLIER papers in this series have been published in the *Transactions of the Royal Society of South Australia*, 1937–1945. The present contribution deals with the morphology of the cercaria and metacercarcia, obtained experimentally, of two Strigeate trematodes, as yet unrecognized as adults. The cercariae were obtained from gastropods living in the swamps of the lower Murray River, South Australia. These are: (1) *Cercaria lessoni* n. sp., from *Planorbis isingi* Cotton, *Limnaea lessoni* Deshayes, and *Simlimnea subaquatilis* Tate; the metacercarial stage occurring in freshwater leeches, *Glossiphonia* spp.; the adult being probably an *Apatemon*. (2) *Cercaria ameriannae* n. sp. from *Amerianna pectorosa* Conrad; the metacercaria occuring in tadpoles of *Limnodynastes* sp. (experimental) and, precociously, in *Amerianna pectorosa;* the unrecognized adult being a Diplostome, perhaps a *Tylodelphys.*

We desire to acknowledge our indebtedness to Messrs. G. G., F., B., and D. Jaensch of Tailem Bend, L. Ellis of Murray Bridge, and W. McAnaney of Lake Alexandrina, for assisting us in obtaining the molluscan material; and to the Commonwealth Research Grant to the University of Adelaide for financial support. Type material has been deposited in the South Australian Museum.

CERCARIA (FURCOCERCARIA) LESSONI n. sp.

(Fig. 1–11.)

A small furcocercaria, *Cercaria lessoni,* has been obtained on a number of occasions from three different species of gastropods, *Planorbis isingi, Limnaea lessoni,* and *Simlimnea subaquatilis.* Eighteen collections of *P. isingi* from the River Murray swamps at Tailem Bend, were made between April, 1937, and February, 1941; the snail was not found again until December, 1945, when it reappeared in large numbers. Further collections of that species were made in January, March and May, 1946, making a total of 3,854 specimens of *P. isingi* collected on all occasions, of which 49 showed infection with *C. lessoni*, i.e. a 1·2 p.c. infection. The rate of infection in *Limnaea lessoni,* as shown by our figures, is a little higher; out of 3,736 snails collected on 43 separate excursions between December, 1937, and May, 1946, 112 specimens gave off *C. lessoni,* i.e. a

2·9 p.c. infection. Two of these collections were made at Swan Reach, the cercaria being obtained on both occasions. *S. subaquatilis* has been found by us in the River Murray swamps only twice: viz. once at Lake Alexandrina, when one out of 208 snails was infected; and once at Tailem Bend, when one out of 46 snails was infected.

The detailed studies have been made entirely with cercariae from *P. isingi*; the larvae from the other hosts were identified later as *C. lessoni* by microscopical examination and measurement.

The cercariae are emitted mainly during the morning, few appearing in the afternoon; the length of life is short, for all are dead within twenty-four hours of emission. The cercariae are actively swimming more than half the time; when resting they are suspended in the water with the furcae spread at an angle of 180°. When few cercariae are present—as in the infections of *P. isingi*, a very small snail—they tend to collect at the bottom of the tube, i.e. the darkest part; in this they resemble *C. pseudoburti* Rankin, which is said (Rankin 1939, 88) to be negatively phototropic. However, infections of *L. lessoni*, a much larger snail, are usually so heavy that a whole test-tube of water may be rendered opaque by the numbers of cercariae emitted within a few hours. The larvae swim with characteristic furcocercarial movement, tail-first, and, if the tube is shaken, vertically upwards. On one occasion two cercariae were observed attached to each other and swimming actively; one was caught on the spines of the other's everted ventral sucker.

For measurement cercariae were fixed by the addition of an equal quantity of boiling 10 p.c. formalin to the water in which they were swimming. Measurements were made with an ocular micrometer, in a water mount, using coverslip pressure only sufficient to keep the cercariae in one plane without distortion. The averages of measurements of ten cercariae, with the range of measurements in brackets, given in micra, are as follows: body length 110 (82–151); body breadth at widest part 31 (27–45); length of tail stem 90 (75–104); breadth of tail stem at widest part 25 (21–28); furca, length 101 (86–113); furca, breadth at widest part 19 (18–21); length of anterior organ 23 (19–27); breadth of anterior organ 18 (16–19); length of ventral sucker 18 (16–19); breadth of ventral sucker 15 (12–18).

In life, the shape of the body varies greatly with the state of contraction, being sometimes squat and nearly round, and at other times greatly elongate (fig. 5). There are no special oral spines. There is a cap of about eight rows of fine spines over the anterior organ, followed by about a dozen irregular rows of smaller spines, the last at the level of the oesophagus (fig. 1). The rest of the body is spineless except for a narrow band of irregularly scattered small spines round the posterior end of the body. No such band is recorded for the related *C. burti* Miller, nor for *C. helvetica XXXI*, in which cercariae the spination is otherwise similar. The ventral sucker is beset with two or three concentric rings of spines alternately arranged and too numerous to count accurately. The opening of the ventral sucker is small and round and leads into a wider bowl-like cavity around the base of which the spines are situated. When the ventral sucker is greatly protruded, the spines are everted through the small aperture and in this condition are at right angles to the body of the cercaria.

The anterior sucker is strongly protrusible; the mouth opening may be inverted deeply into it, or pushed forward when the anterior organ is protruded. The straight narrow prepharynx leads into a well developed pharyngeal bulb. The rest of the digestive tract is difficult to see. Just anterior to the ventral sucker the narrow oesophagus bifurcates into two short deeply constricted caeca, the last lobes of which appear to be completely separated from the rest of the intestine. This condition resembles that described by Sewell (1922, 277) for *C. indica XXII*, by Miller (1926, 43) for *C. burti*, and by Wesenberg-Lund (1934, 114) for *C. longiremis*. The ends of the caeca, which scarcely extend beyond the posterior border of the ventral sucker, stain with intra-vitam neutral red, but not with intra-vitam Nile blue sulphate.

Dorsal to the caeca on either side lie four small granular penetration cells (fig. 1) one behind the other, the first antero-lateral to, the last postero-lateral to the ventral sucker. The cells stain deeply with Nile blue sulphate, lightly with neutral red in strong solution, and are unstained in Orange G solution. The cells are granular with large, clear, non-staining nuclei. The proximal part of each duct is granular, the rest clear; they pass forward to penetrate the anterior organ laterally and open on either side of the mouth. The gland-cells are difficult to see in living specimens except when deeply stained; in this respect they are apparently similar to those of *C. helvetica XXXI* which Dubois (1929, 94) described as "peu distinctes." In preserved specimens the gland-cells stained with neither acid alum carmine nor Delafield's haematoxylin. Antero-lateral to the ventral sucker on each side is a clear roundish refractory body (fig. 1), which did not stain with any stain used. These bodies are apparently "unpigmented eyespots," such as those described for *C. pseudoburti* by Rankin (1939, 89) and for *C. ranae* by Cort and Brackett (1938, 264).

A thick band of nerve fibres staining with intra-vital Nile blue sulphate lies dorsal to the oesophagus just behind the pharyngeal bulb (fig. 1). The genital primordium is a mass of cells staining deeply with acid alum carmine and Delafield's haematoxylin; it lies between the ventral sucker and the bladder (fig. 1).

The tail is longifurcate, with a border of fine, short spines along each side of

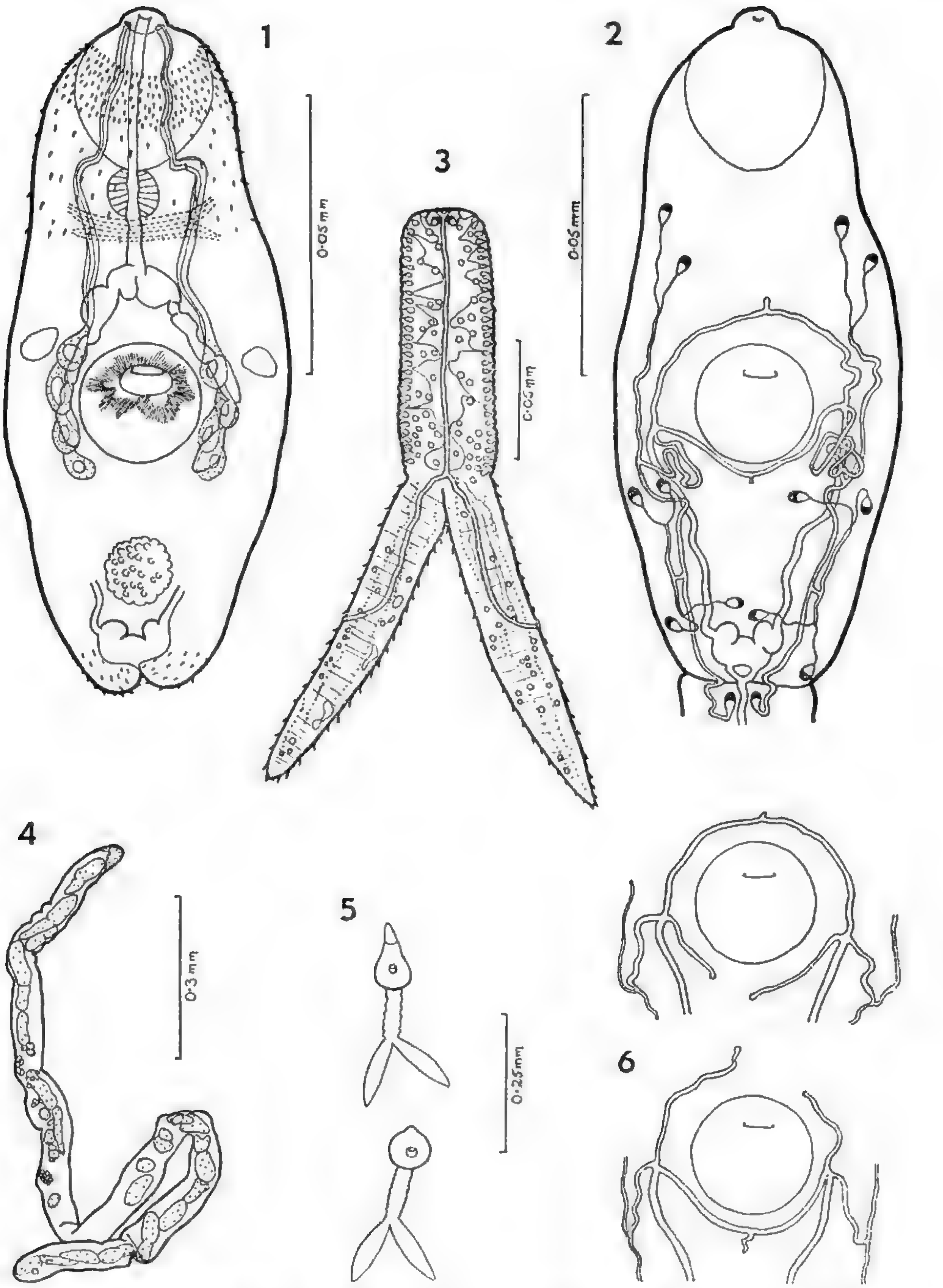


Fig. 1–6, *Cercaria lessoni*. 1. glandular, nervous, digestive and reproductive systems, and spination; 2. excretory system; 3. tail; 4. sporocyst; 5. showing different shapes assumed according to the state of contraction; 6. two variations in the excretory commissures. Outlines of fig. 1–5 drawn with camera lucida; fig. 6 drawn freehand.

the furcae (fig. 3). There are five or six irregular pairs of large clear cells, variable in shape, within the tail-stem. These "caudal bodies" are anchored to the sides of the tail-stem by fine strands of tissue, and are also attached to the central excretory canal of the tail. Numerous small stalked cells line the sides of the tail-stem, their bodies projecting inwards and moving freely. Longitudinal and transverse muscle fibres are well-developed.

The body of the bladder (fig. 2) is rounded, with a bulge on either side from which the main collecting tube passes forward to the level of the posterior border of the ventral sucker. Here it takes a sharp bend posteriad, continuing for a short distance as a greatly convoluted tube which receives two secondary tubules, one from the anterior part of the body, draining one pair of flame-cells, the other from the posterior part of the body, draining two pairs of flame-cells in the body and a single flame-cell in the anterior extremity of the tail-stem, the formula being $2[(2) + (2 + 2 + (1))] = 14$.

Where the main excretory tube bends posteriad, it receives two transverse commissures, one anterior to, the other posterior to, the ventral sucker. The extent to which these commissures are developed varies considerably in different specimens. The commissures arise as a pair of branches growing out from the main excretory duct on either side, at the point where this duct turns posteriad. The posterior branch from each side grows towards the mid-line; the anterior pass forward by the side of the ventral sucker before growing inward to meet. In any one cercaria, both pairs of commissural outgrowths, or one only (either the anterior or the posterior), or neither, may meet and fuse, the point of fusion being as a rule the point of origin of a short, blindly-ending vessel. Thus four stages of fusion have been observed, and in cases where fusion has not occurred, the degree of development of the commissures is very variable (fig. 6).

Posterior to the bladder is an island of Cort, and from this the excretory canal of the tail passes posteriad along the tail-stem, branching at the origin of the furcae into two tubes, each of which opens by a pore to the exterior halfway along the anterior border of the furca.

SPOROCYST.

Upon dissection of a host *Planorbis*, the liver was found to be almost completely replaced by narrow, elongate, colourless sporocysts with rounded ends (fig. 4). The length of the longest sporocyst dissected out entire was 3 mm. (preserved in formalin), i.e. they are relatively small. No birthpore was observed; cercariae and germ-balls are scattered without order along the length of the parasite. The wall of the sporocyst is studded with cells which stain with haematoxylin; the cuticle is yellowish and faintly wrinkled. Living sporocysts are capable of slight movement.

Sporocysts taken from the liver of *L. lessoni* infected with *C. lessoni* were similar, but were present in far greater numbers as the latter snail is many times larger than *P. isingi,* and is subject to very heavy infestations with this parasite.

METACERCARIA.

Attempts have been made to infect with *C. lessoni*, the fish *Gambusia affinis* and *Carassius auratus*; tadpoles (*Limnodynastes* sp. and *Hyla peroni*); molluscs (*Amerianna* spp., *Limnaea lessoni*, *Planorbis isingi*); the yabby (*Cherax destructor*); and mosquito larvae. None of these attempts was successful. On two separate occasions, about six weeks apart, two leeches (*Glossiphonia* sp.) were both exposed to fairly heavy infections; seven weeks after the second exposure the leeches were dissected. Both contained a large number of thick-walled cysts, of two sizes, embedded in the tissues of the body wall. As the leeches used had been taken from the River Murray, and had thus been exposed to the possibility of natural infection, the results of the experimental infection are open to question; but the occurrence of the cysts in two sizes, corresponding with the two infections, and the large number recovered, indicate that they were the result of experimental infection. The same sort of cysts were recovered from a similar leech exposed to infection with cercaria from the other host, *Limnaea lessoni*.

The cysts (fig. 8) are thick-walled and slightly egg-shaped. The measurements (in micra) of a cyst of the smaller size, are as follows: length of cyst, 295; breadth of cyst, 246; length of cavity of cyst, 205; breadth of cavity, 180. Measurements of a cyst of the larger size are: length of cyst, 393; breadth, 328; length of cavity, 278; breadth 246.

The thickness of the cyst wall made the excystment of the living metacercaria unfeasible by ordinary mechanical methods; so a solution of pepsin in 0·4 p.c. hydrochloric acid, warmed slightly, was used to dissolve the cyst wall and liberate the metacercaria. Although this ensured that undamaged metacercariae were obtained, none remained alive long enough to facilitate study of the excretory system, so that only the grosser features were seen (fig. 7). Further study was made using preserved specimens, stained with neutral red and examined in a serum mount, or stained with Delafield's haematoxylin and examined in methyl salicylate mounts.

The metacercaria is a Tetracotyle, fairly active in life, especially when warmed slightly, as in the process of dissolving the cyst. In a cyst under cover-slip, most of the anatomical features of the larva can be seen, although the proportions of the body cannot be determined (fig. 10). In an excysted metacercaria (fig. 7 and 9), the bipartite nature of the body is obvious, the posterior part containing the genital primordia being much smaller than the anterior containing the organs of adhesion. These comprise two lateral suctorial cups (fig. 7),

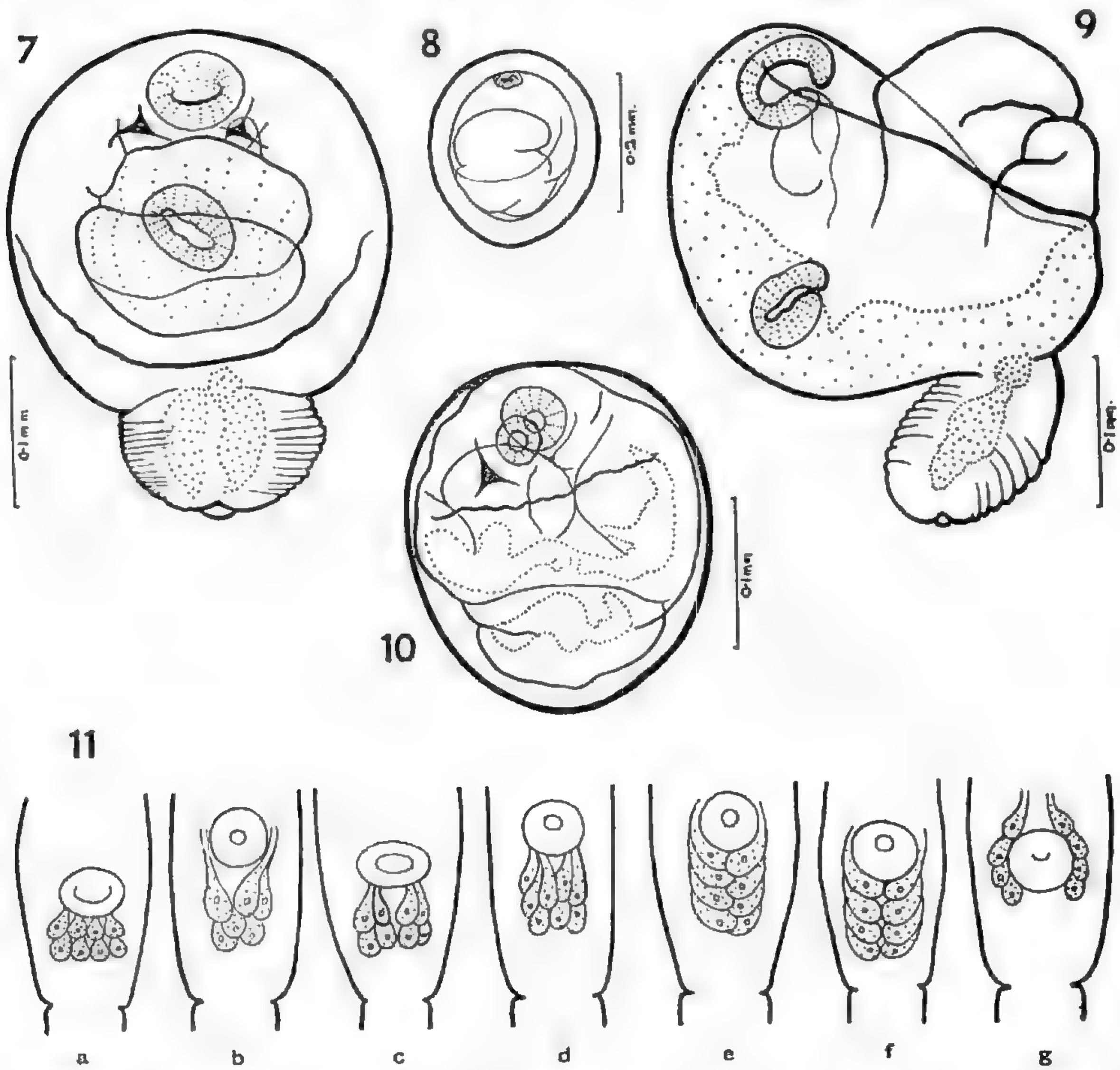


Fig. 7–11, *Cercaria lessoni.* 7. metacercaria freed from cyst, ventral view; 8. cyst; 9. metacercaria, lateral view; 10. metacercaria within cyst, ventral view (inner wall only of cyst shown); 11. a number of *Apatemon* cercariae arranged in series according to the disposition of the gland-cells—a, Cercaria of *Apatemon gracilis* (after Szidat); b, *C. riponi* (after Brackett); c, *C. pseudoburti* (after Rankin); d, *C. burti* (after Miller); e, *C. helvetica XXXI* (after Dubois); f, *C. pygocytophora* (after Brown); g, *C. lessoni.*

and two large suctorial lips enclosed in a ventral pocket. The anterior and ventral suckers are well-developed. A pharynx was not seen, but in figures of the closely similar Tetracotyle of *Apatemon gracilis*, described by Szidat (1931, 143–4), a very faint pharynx is indicated.

RELATIONSHIPS.

Cercaria lessoni is a longifurcate, distome, pharyngeal cercaria whose nearest relative is *C. helvetica XXXI* (Dubois, 1929) from *Limnaea* and *Planorbis*. The measurements of the latter (body length, 120–140; tail-stem, 135–155; furca length, 160–180) show it to be a little larger; but it is characteristic of this type of cercaria is to be "very contractile" (Dubois 1929, 95), as the range of measurements of *C. lessoni* indicate, so that this difference is probably of little importance, especially as Dubois' method of fixing cercaria may have been different from ours. The shape and proportions of the body are closely similar; the excretory systems are identical, except that, in *C. lessoni*, the commissures are not always completely developed, and that the flame-cells in the caudal stem are placed higher than those of Dubois' cercaria. The nature of the intestinal caeca is apparently the same in both; those of *C. lessoni* were exceedingly difficult to see, and Dubois was unable to follow those of *C. helvetica XXXI* to their termination. The most conspicuous difference is in the gland cells; in Dubois' cercaria they are disposed in two longitudinal series, each of four cells, posterior to the ventral sucker, and are somewhat larger than those of *C. lessoni;* while in the latter, the two longitudinal series have become pushed forward and lie on either side of the ventral sucker. The gland cells of both have the same character, however, being very indistinct. The only other differences are: firstly, that unpigmented eyes are not recorded for Dubois' cercaria, but these are in any case not conspicuous features; secondly, that no mention is made of minute scattered spines around the posterior part of the body, as in *C. lessoni;* and finally, that no island of Cort is indicated, but this again is a very inconspicuous feature.

Another cercaria, *C. pygocytophora* Brown (1931), from *Planorbis*, is very closely allied to *C. helvetica XXXI*, and to *C. lessoni*. The flame-cell formula is the same, and spination, caudal excretory system, body size and shape are all practically identical with those of the other two cercariae. The glands, eight in number, are arranged like those of *C. helvetica XXXI*. Apart from the latter difference, the only other distinctions between *C. pygocytophora* and *C. lessoni*, are the presence of hair-like structures on the tail-stem of the former, and the nature of its excretory commissures. The latter are apparently rather less completely developed even than those of *C. lessoni;* no trace of a posterior commissure is shown, while the branches of the anterior have not met in the cercarial stage.

But for the presence of the commissure anterior to the ventral sucker, *C. lessoni* resembles closely another group of cercaria, which includes the cercaria of *Apatemon gracilis* (from *Bithynia*), *C. burti* Miller (from *Limnaea* and *Planorbis*), and *C. pseudoburti* Rankin (from *Limnaea*). These have only one commissure behind the ventral sucker, but have an excretory formula identical with that of *C. lessoni*. The size, shape, proportions and spination are similar. All have four pairs of penetration glands, but these are behind the ventral sucker, arranged either in two horizontal rows, as in the cercaria of *Apatemon gracilis*, or in two groups of four, as in the others. Long, fine hair-like processes, like those of *C. pygocytophora*, are shown on the sides of the tail-stem of the cercaria of *A. gracilis*, but no such processes were observed on *C. lessoni*. Eyes are not recorded for *C. burti*. *C. pseudoburti* is very similar to *C. lessoni*, but in the former, the lack of anterior commissure, the absence of spines at the posterior end of the body, as well as the disposition of the gland-cells, serve to distinguish it from our cercaria.

To this group might be added *C. riponi* Brackett, 1939 (from *Stagnicola*) which agrees with the descriptions of the others, except that the glands, arranged like those of *C. burti* and *C. pseudoburti*, in two groups behind the ventral sucker, are given as only six in number. If these glands are of the same indistinct nature as those of *C. helvetica XXXI* and *C. lessoni*, the difficulty encountered in distinguishing the precise number would be considerable.

A number of other cercariae appear to have some affinities with *C. lessoni*, though not so closely related as the six mentioned above. The cercaria of *Apharyngostrigea pipientis* Olivier (1940), from *Planorbula*, has eyespots and four pairs of gland-cells rather like those of our larva; it also has two excretory commissures, though their relation to the rest of the excretory system is different. The excretory system formula is $2[(2+2)+(2+2+(2))]=20$, i.e., it shows development of a greater complexity with the same pattern as that of *C. lessoni* and its closest relatives. In this connection it is interesting to note that the flame-cells in the tail of the cercaria of *A. pipientis* are in the same position as those of the other group, and that the two flame-cells of each side are very closely connected, as though their division is very recent. The size of the cercaria is of the same order, but it is very obviously distinguished from *C. lessoni* by the peculiar nature of its tail, as well as by minor features such as spination.

The cercaria of *Apharyngostrigea ibis* Azim 1935 (from *Planorbis, Physopsis* and *Pyrgophysa*) is less similar to *C. lessoni* in some respects than the cercaria of *A. pipientis*. The shape and size of the body are of the same order, and the spination probably similar, though the hair-like processes on the tail are a distinction, and no pharynx is shown in Azim's figure. There appear to be, not four, but three pairs of gland cells arranged as in the cercaria of *A. pipientis*. The furcal excretory tubes open at the tips of the furcae, unlike those of *C. lessoni* and the related forms; the excretory system, so far as can be determined from the diagram, possesses no commissures; and though the flame-cells are fourteen in number, with two single flame-cells in the tail stem, the formula is different from that of *C. lessoni*. The metacercaria of both these forms is found in tadpoles.

C. dohema Cort and Brackett (hosts, *Stagnicola* and *Limnaea*) resembles our cercaria in size, spination, presence of eyespots and a posterior excretory commissure, and in the excretory tubes of the tail, which open half-way along the furcae; but the excretory system though of the same fundamental pattern, is less complex, its formula being $2[2 + (2 + (1))]$; there are only six glands lying behind the ventral sucker; the digestive system is greatly reduced. *C. angelae* Johnston and Simpson 1944 (from *Ameriauna*), has somewhat similar spination, two groups of four gland-cells, in tandem, an excretory commissure behind the ventral sucker, and a single pair of flame-cells high in the tail stem; but the grouping of the flame-cells in the body is different, and the furcal excretory tubes open at the tips. The excretory system of *C. bulbocauda* Miller (from *Planorbis*) appears to be identical with that of *C. lessoni*, but the nature of the tail and digestive system excludes it from immediate relationship. *C. hirsuta* Miller and *C. granula* Miller (both from *Planorbis*) exhibit some features in common with our cercaria, including excretory systems of similar but not identical formulae; but both forms possess a greatly reduced digestive system, and a very large number of small glands behind the ventral sucker.

C. furcicauda Faust, *C. robusticauda* Faust, and the cercaria of *Neodiplostomum lucidum* (La Rue and Bosma) have each a single flame-cell pair high in the tail, and excretory pores halfway along the furcae, but in none of these three is the arrangement of the flame-cells exactly similar to that of *C. lessoni*, while the absence of a posterior commissure, and the number and arrangement of the gland-cells, indicate that, though some relationship is possible, it is not close.

C. gracillima Faust (from *Physa* and *Limnaea*), *C. bdellocystis* Lutz (from *Planorbis*) and *C. longiremis* Wesenberg-Lund (from *Valvata*) may be related species, but the descriptions are inadequate to verify this. *C. bdellocystis* is said to encyst in leeches and develop into *Apatemon bdellocystis* (in pigeons, experimentally).

Furcocercaria I Petersen (from *Limnaea* and *Physa*) has two groups of three gland-cells, and an excretory commissure posterior to the ventral sucker; the body proportions, spination and contractility are similar to *C. lessoni*, but the excretory system as indicated in the diagram shows a number of differences. *C. secobii* Faust (from *Limnaea* and *Physa*) has four pairs of gland-cells in tandem but, though the excretory system is not known, the large size of the tail makes close relationship with *C. lessoni* improbable.

C. ranae Cort and Brackett has eyespots and a commissure more or less behind the ventral sucker, but is distinguished from our cercaria by the gland-cells, spination, and the flame-cell formula. *C. obscurudena* Brackett has only a single pair of flame-cells high in the tail-stem, but the rest of the excretory system and gland-cells exclude it from close relationship.

C. indica I Sewell (from *Indoplanorbis*) possesses spination and a caudal excretory system like that of *C. lessoni*, but the body excretory system differs somewhat, there is no posterior commissure, and there are only two pairs of gland-cells before the ventral sucker. Concerning *C. indica XXII*, Sewell mentioned the curious nature of the intestinal caeca, a feature characteristic of *C. lessoni;* it is, however, the only feature they have in common, for *C. indica XXII* has four flame-cells, well-spaced out, in the caudal trunk—a characteristic of *Cotylurus* and Diplostome cercariae, and very different from the condition in *C. lessoni* and its closest allies.

DISCUSSION.

It is apparent that the affinities of *C. lessoni* are mainly with the six cercaria first mentioned, i.e. *C. helvetica XXXI*, *C. pygocytophora*, the cercaria of *Apatemon gracilis*, *C. burti*, *C. pseudoburti*, and perhaps *C. riponi*. In 1938, Willey and Rabinowitz (1938) proved *C. burti* to be the larva of *Apatemon globiceps*, the metacercaria being a Tetracotyle occurring in leeches. The metacercariae of *C. helvetica XXXI*, *C. pseudoburti*, *C. pygocytophora* and *C. riponi* have not been described, though it is known that *C. pseudoburti* does not encyst in nymphs of mayflies and dragonflies, gammarids, tadpoles, fish or mice. Dubois, in discussing the genus *Apatemon* (1938, 96) mentioned *C. helvetica XXXI* as an *Apatemon* larva distinct from that of *A. gracilis*, whose cercaria was at that time known, and whose tetracotyle had been described from leeches by Szidat (1929; 1931). Lutz's *Dicranocercaria bdellocystis* (1934) appears to be a further example of an *Apatemon* cercaria encysting in leeches. Although no other complete life cycles have been established for the genus *Apatemon*, two other *Apatemon* metacercaria have been described from fish—the tetracotyle of *Apatemon fuligulae* by Yamaguti, encysting in Siluridae, and the tetracotyle of *A. pellucidus*, encysting in *Mogurnda*.

The cercariae listed above, together with *C. lessoni*, form a particularly well-defined group of seven cercariae (if *C. riponi* is included) so closely allied that their differences are unlikely to be more than inter-specific. As a group, these "*Apatemon* cercaria" are characterized by certain features which distinguish them clearly from cercariae of related genera, such as those of *Cotylurus* and *Diplostomum*. The *Apatemon* cercariae are extremely small and very active. The body is characteristically pear-shaped, but may assume a variety of forms because of the remarkable contractility of the body; both Miller (1926, 41) and Dubois (1929, 95) comment on the great extensibility of the forms they describe.

The spination is sparse, mainly confined to the anterior part of the body. The pharyngeate alimentary canal ends in caeca which are short, faint and may be lobed. The penetration glands are eight in number, except in the doubtful *C. riponi*; these glands are usually behind the ventral sucker. The variations in their arrangement constitute an important means of species identification. When arranged in order according to the disposition of their gland-cells, the cercariae form a fairly definite series, from the cercaria of *Apatemon gracilis*, with glands in two horizontal rows, through *C. pseudoburti* and *C. burti*, with glands more longitudinally disposed in clusters, to *C. helvetica XXXI*, *C. pygocytophora* and *C. lessoni*, in which the glands have become drawn out into two longitudinal rows (fig. 10).

Perhaps another characteristic of the *Apatemon* cercariae is the presence of unpigmented eyespots, though they are not described in all. The tail is always longifurcate; the tail-stem, body and furcae are of much the same length in each cercaria. The flame-cell formula is $2[2 + (2 + 2 + (1))] = 14$. The presence of only two flame-cells in the tail-stem, instead of four, as is usual in *Cotylurus* and Diplostome cercariae, and their position immediately behind the junction of body and tail is an invariable feature. Sewell (1922, 267) in discussing *C. indica I*, sole member of his *Pahila* group, considers that the presence of a single flame-cell pair in the tail-stem is probably a group character rather than a specific difference, a view which this set of cercariae tends to support; but the feature also characterizes another group, Miller's *Elvae* group of ocellate cercariae, which are not otherwise closely related, as well as various other cercariae, mentioned above, which are not closely allied to the *Apatemon* cercariae. This character is hence merely indicative and not diagnostic until considered with the other special features. Likewise, the position of the excretory pores of the tail, half-way along the furcae, is typical not only of the *Apatemon* cercariae, but also of many *Cotylurus* and *Diplostomum* cercariae.

The early development of parts of the reserve excretory system is a further characteristic of the *Apatemon* cercariae. This allies them to the *Cotylurus* group, in contrast with the Diplostome cercariae. In the former, the commissure usually developed is that anterior to the ventral sucker; in the *Apatemon* group, four of the seven cercariae possess one commissure, posterior to the ventral sucker, while the other three (*C. helvetica XXXI*, *C. pygocytophora* and *C. lessoni*) display variability in the extent to which the commissures are developed. *C. helvetica XXXI*, the most precocious, has two fully developed commissures; *C. pygocytophora* shows least development, with only an anterior commissure, incompletely developed; while in *C. lessoni* growth and fusion of the two commissures takes place to a variable extent in cercarial life. Precocious development of the reserve system has been observed in a number of cercariae, e.g. *C. sanjuanensis* Miller, in which blind tubes arise from the commissure. Olivier (1940, 463), in mentioning the well-developed commissures in the cercaria of *Apharyngostrigea pipientis,* points out that "the presence or absence of transverse commissures in cercaria simply indicates differences in the rate of development of their respective excretory systems." The differences in rate of development are, however, group characteristics and are useful diagnostically; and Dubois (1944, 81) states that the diverse genera of Cotylurini and Strigeini represent forms more highly evolved than the Diplostomini, since in the former, the anastomosing processes have already appeared in the cercaria, while in the latter, they are only developed in the metacercaria.

Finally, *Apatemon* cercariae are limited in their host range to species of *Limnaea, Planorbis* and *Stagnicola,* except for the cercaria of *A. gracilis,* whose host is *Bithynia*; and the metacercaria is a Tetracotyle which forms a thick-walled, slightly oval cyst.

We may point out that a species of *Apatemon, A. intermedius* (S. J. Johnston) Dubois, has been described from the black swan, *Chenopis atrata,* which is a common bird on the Murray swamps.

CERCARIA (FURCOCERCARIA) AMERIANNAE n. sp.

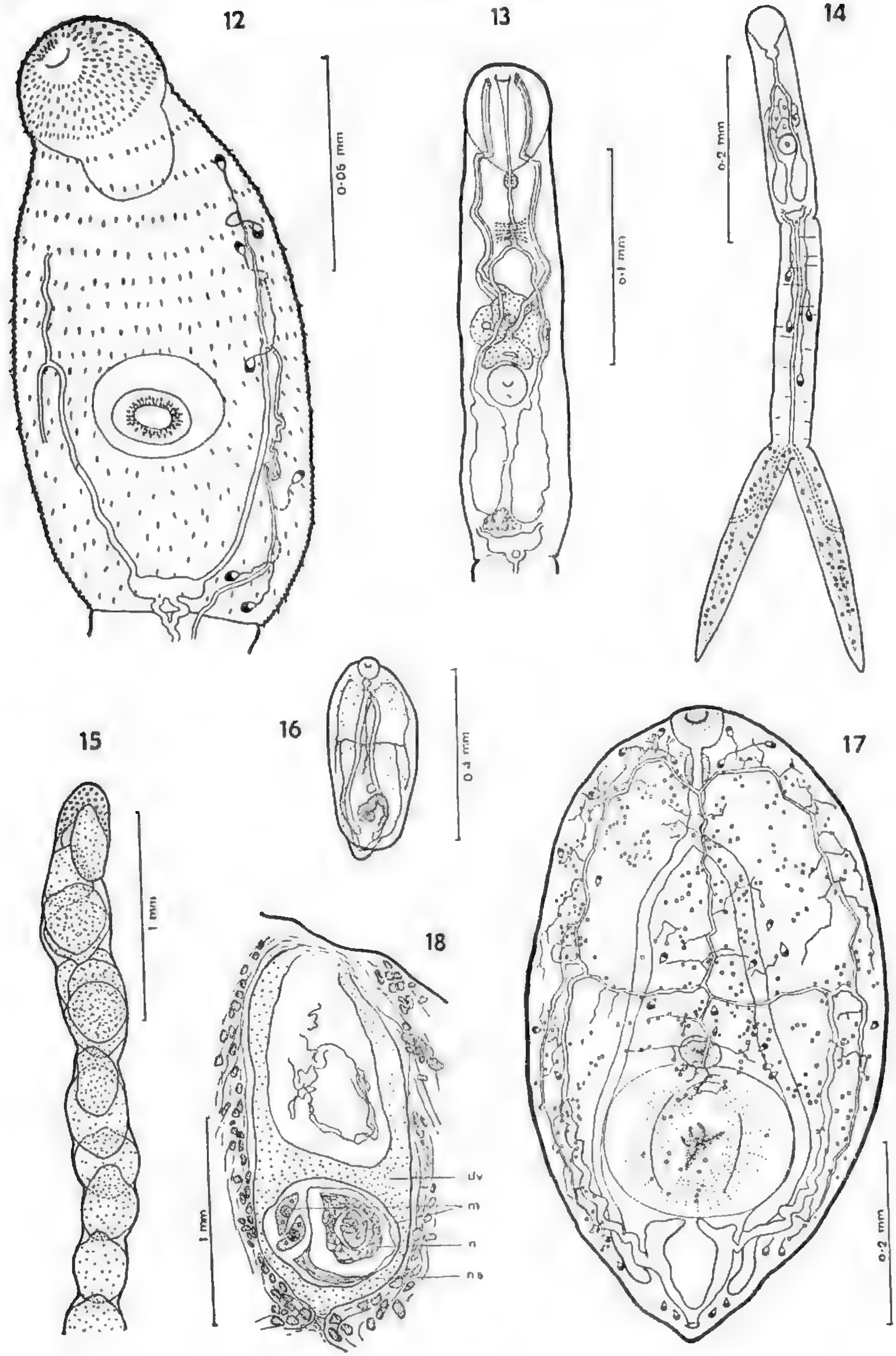
(Fig. 12–18.)

A new cercaria, *C. ameriannae* has been obtained on two occasions only, in both cases from *Amerianna pectorosa* collected from the Murray swamps at Tailem Bend. It was found in one of 166 *Amerianna* spp. collected in October, 1944, and in one of 400 in December, 1946. It is evidently a rare larva in that locality since it has not been observed on any other occasion during our ten-year survey of the molluscan parasites of the region.

The cercariae are emitted mainly during early morning. They are not particularly active, spending most of the time suspended in the water with the furcae spread at about 180°. They swim in characteristic furcocercarial fashion. The length of life was not determined.

The averages in micra, of ten measurements, based on specimens fixed as indicated earlier, followed by the range in brackets, are as follows: length of body, 209 (180–229); breadth of body at widest part, 40 (30–49); length of tail-stem, 259 (229–295); breadth of tail-stem at widest part, 34 (32–41); length of furca, 243 (131–287); breadth of furca at widest part, 20 (16–32); length of anterior organ, 45 (37–59); breadth of anterior organ, 29 (27–32); length of ventral sucker, 22 (21–27); breadth of ventral sucker, 22 (19–27).

In life, the body when greatly contracted (as in specimen drawn in fig. 12),


12
0·05 mm
13
0·1 mm
14
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15
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0·1 mm
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is striated transversely by wrinkles, but these disappear when the body is extended, as in fig. 13.

In front of the mouth are about thirteen fine spines, arranged alternately in two rows (fig. 12). There is a small circumoral spineless area, followed by a wide collar of 9–10 rows of spines, covering the anterior organ. Behind this the body is entirely covered with spines, arranged in rows over the anterior half of the body, and irregularly scattered more posteriorly. The ventral sucker is beset with about seventy long slender spines arranged in two concentric rings. There is a single row of fine spines along each side of the furcae.

The mouth opening (fig. 13) may be pushed forward or withdrawn deep into the anterior organ. Behind the latter is a short prepharynx followed by a stout muscular pharynx, which is succeeded by a narrow oesophagus. The latter bifurcates to form the two very long intestinal caeca. These are more than half as long as the whole body; the longer anterior portion is narrow, but in the region of the ventral sucker they widen suddenly into two pouches which extend back to the anterior border of the bladder. The wide part of the caeca stains faintly with intra-vitam neutral red.

Immediately in front of the rather small ventral sucker, and ventral to the caeca, lie four large prominent penetration gland-cells, the cytoplasm of which stains with neutral red used intra-vitam. The highly characteristic arrangement of these is shown in fig. 13. The ducts of the four cells travel forwards, a pair on either side, and enter the anterior organ, the point of entry being marked by a constriction in their diameter. The ducts open beside the mouth.

Dorsal to the oesophagus, behind the pharynx, lies a transverse band of nervous tissue. The genital primordium consists of a small, more or less triangular mass of cells, situated between the ends of the caeca and the bladder.

The longifurcate tail is very large relative to the body (fig. 14); both tail-stem and furcae are longer than the body. The tail-stem is very muscular; it contains no caudal bodies.

The bladder is broad and slightly lobed at each lateral extremity. Each lobe receives a main collecting duct which passes forward, without coiling, to the level of the anterior border of the ventral sucker, where it receives two secondary tubules, an anterior, draining two pairs of flame-cells (fig. 12), and a posterior, draining a single flame-cell and one pair in the body, and a further pair, well

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Fig. 12–18, *Cercaria americannae*. 12. spination and excretory system of body; 13. glandular, nervous, digestive and reproductive organs; 14. general proportions of cercaria, and excretory system of tail; 15. part of a sporocyst; 16. metacercaria, uncompressed; 17. anatomy of metacercaria; 18. section through dorsal body wall of tadpole showing metacercariae in situ in the notochord. Outlines of all figures were drawn with a camera lucida.

dv = developing vertebra of tadpole; m = metacercaria; n = notochord; ns = notochordal sheath.

spaced out, in the tail-stem (fig. 14). The flame-cell formula is thus $2[(2+2)+(1+2+(2))]=18$. The posterior tubule is also furnished with two patches of cilia, just after its origin from the main duct. From the posterior border of the bladder the central excretory canal of the tail arises; it forms an island of Cort at the junction of body and tail-stem, and then passes down the tail-stem to divide at the origin of the furcae into two ducts. The opening of these, as far as could be determined from preserved material, probably occurs halfway along the furcae (fig. 14).

Sporocyst.

The single host snail of this parasite lived in the laboratory for nearly three months. Upon death it was dissected; most of the liver had been replaced by masses of tangled, whitish sporocysts. The first attempt to disengage one of these liberated an immense number of actively moving diplostomula, and every sporocyst was packed with them (fig. 15), but no cercariae were seen, although the latter were being emitted only twelve days earlier. The sporocyst itself consisted of nothing more than a delicate tubular skin around the diplostomula. A long sporocyst measured 5 mm.; but most of them were shorter.

Precocious development of diplostomula within the parent sporocyst has been recorded for various species, including *C. metadena* Johnston and Angel (1942), observed in this laboratory. In the report on this observation, an account was given of other observations of this phenomennon, which is apparently confined to the Diplostomes, and also a discussion of the possible causes of such precocious development.

Metacercaria.

The fish, *Gambusia affinis*, resists experimental infection with *Cercaria ameriannae*. Tadpoles of *Limnodynastes* sp., probably *L. dorsalis*, are however highly susceptible to infection. Two of these were placed in contact with large numbers of cercariae. On the following day both tadpoles were dead, and microscopical examination showed numerous tail-less cercariae moving through the tissues. Lighter infections employed subsequently enabled diplostomula of considerable size to be raised. They do not secrete a cyst wall, and for some time after penetration are to be found wandering in the tissues. Eventually they penetrate the notochord, and develop within it in numbers. Sections show that, where they penetrate, the notochord becomes hollowed out. Usually a group of about half-a-dozen are found together.

The diplostomula are very active when alive, and are very extensible—there may be a difference of 82μ between the length when extended and the length when contracted. The averages of measurements of six preserved metacercaria, given in micra, with the range in brackets, are as follows: length of body, 377 (319–410); breadth of body at widest part, 221 (205–237); length of anterior organ, 36 (30–37); breadth of anterior organ, 34 (30–36); length of pharynx, 29 (28–30); breadth of pharynx, 25 (23–27); length of holdfast, 79 (63–95); breadth of holdfast(81 (66–90). The ventral sucker was not measured as it was very indistinct in most specimens. The six specimens measured were taken from a tadpole infected ten months previously.

When the diplostomulum is uncompressed (fig. 16), it may be seen that the body is composed of two portions, a large leaf-like anterior part, and a small posterior region. The anterior part is slightly concave ventrally and is furnished with a well-developed holdfast, beset ventrally with minute spines (fig. 17). The posterior part contains the bladder and genital primordium.

The anterior part has grown considerably more than has the acetabulum, which is situated immediately in front of the holdfast organ. The pharynx is stout and muscular, leading to a short oesophagus, followed by a pair of long slender caeca, extending to the posterior part of the body; the bulging ends, which characterized the cercarial stage, have not persisted.

The bladder is composed of two long horn-like branches, and has a single excretory pore on its posterior border. From each branch of the bladder a a stout collecting duct passes forwards on each side. Just in front of the level of the ventral sucker each duct receives a short collecting tube into which open, firstly, a posterior tubule, passing back to the level of the bladder and receiving numerous lateral capillaries; and secondly, an anterior tubule also draining numbers of capillary tubes; this anterior tubule passes forwards to the level of the pharynx where it bends mesiad and fuses with its fellow from the opposite side. From the point of fusion a central vessel passes posteriad, receiving many side-branches. In front of the ventral sucker it is joined by a commissure which passes from the branching end of one main collecting duct to the other. The central vessel continues posteriad to the region of the holdfast, where it ends in branches.

The flame-cells have increased considerably in number; a complete study was impracticable but the position of a number is indicated on the diagram. Numerous refringent excretory granules are present throughout the body, connected with the newly developed capillary channels of the excretory system.

RELATIONSHIPS.

C. ameriannae is a Strigeid larva of the longifurcate, pharyngeal, distome type. Since it possesses no excretory commissure, it is at once distinguished from the *Apharyngostrigea*, *Apatemon*, and *Cotylurus* cercariae; the only notable point of similarity between *C. ameriannae* and most of the last-named group is the possession of four pre-acetabular gland-cells, and two pairs of flame-cells in the tail. This last characteristic distinguishes *C. ameriannae* at once from such cercariae as *C. indica I* Sewell, *C. furcicauda* Faust, and *C. Neodiplostomi-lucidi* (La Rue and Bosma), which, though possessing four gland-cells, have only two flame-cells in the tail, near the junction of the body.

Our cercaria is rather more closely allied to a number of cercariae which, besides possessing four gland-cells, have several other significant features in common with *C. ameriannae*. *C. chrysenterica* Miller, from *Limnaea megasoma*, is among these. The body of this cercaria is very slightly larger, but the proportions are similar. As in *C. ameriannae*, the caeca are distended posterior to the ventral sucker. The spination on both is similar. The genital primordium of each is wedge-shaped. The excretory systems differ in one point, for, though there are fourteen flame-cells in the body and four in the tail in each, the formula for *C. chrysenterica* is $2[(2+1)+(2+2+(2))]=18$, instead of $2[(2+2)+(2+1+(2))]=18$ as in *C. ameriannae*. Other differences are in the caudal bodies: *C. ameriannae* has none, whereas *C. chrysenterica* has several small irregular ones; and in the gland-cells which, though ventral to the caeca, four in number, and of similar size to those in *C. ameriannae*, are post-acetabular in position. They are, however, arranged in a grouping rather similar to that of the gland-cells in our cercaria. The second intermediate host of *C. chrysenterica* is unknown.

C. sudanensis No. 5 (Archibald and Marshall), from *Bulinus*, may be even more closely allied to *C. ameriannae*. It is unfortunate that its excretory system has not been described, for in all other respects, and most particularly with regard to the shape of the caeca, it resembles *C. ameriannae* very strongly, as far as can be determined from the description and figure. The major difference is that the two median gland-cells are situated slightly anterior to the two lateral cells. This arrangement is seen again in *C. letifera* (Fuhrmann), from *Limnaea*; but in this cercaria, the caeca are shorter and dilate less abruptly than in *C. ameriannae*, there is one fewer flame-cell on each side (the formula is $2[(2+2)+(2+(2))]=16$), and there are well-developed caudal bodies. *C. tenuis* Miller has four pre-acetabular gland cells with the same arrangement, but the caeca are shorter and are not dilated, and the flame-cell formula is $2[(2)+(2+1+(2))]=14$.

C. Diplostomi-spathacei (Rudolphi) (= *C. helvetica XXXI* Dubois), *C. Diplostomi-flexicaudi* Cort and Brooks, and *C. Diplostomi-murrayensis* Johnston and Cleland, all from species of *Limnaea*, are related to *C. ameriannae*. The important differences in these cercariae are firstly, that in all three the glands, though ventral to the caeca and exhibiting a tendency to be arranged as in *C. amerianmae* are posterior to the ventral sucker; secondly, that there is one fewer flame-cell on each posterior collecting tubule, the formula being either $2[(2+1)+(2+1+(2))]=16$, or $2[(2+1)+(1+2+(2))]=16$; and thirdly, that caudal bodies are present in the tail-stem. In none do the caeca suddenly distend.

C. yogena Cort and Brackett, from *Stagnicola*, is very similar structurally to the last three, though slightly smaller; like them it has four gland-cells behind the ventral sucker, caudal bodies, and the same excretory formula. *C. maritzburgensis* Porter from *Limnaea*, is another form closely related to *C. yogena*, possessing the same excretory formula, and differing mainly in certain details of spination and proportions of the tail.

C. micradena Cort and Brackett (from *Stagnicola*), the larva of *Diplostomum micradenum*, with its metacercaria in tadpoles, and *C. tetradena* Johnston and Beckwith (1945), from *Plotiopsis*, are both smaller than *C. amerianmae*, but resemble it closely in a number of points; they differ in the shape of the caeca, the arrangement of the gland-cells, and in the possession of one more flame-cell on each posterior tubule. In *C. micradena* also the gland-cells are very small. *C. macradena* Cort and Brackett, from *Stagnicola*, which has the same excretory formula as *C. micradena*, is of a size similar to that of *C. amerianmae*, but the spines are nowhere arranged in definite transverse rows, the gland-cells are much larger than those of *C. amerianmae* and are situated differently, and the four flame-cells in the tail are situated close to the junction of tail and body.

C. longifurca Cort and Brooks, from *Limnaea*, and *C. marcianae* Cort and Brooks, from *Planorbis*, each with two small pairs of gland-cells, differ markedly in spination, size (both are much smaller than *C. amerianmae*), shape of the caeca, and proportions of the tail, and in the possession of either one (*C. longifurca*) or three (*C. marcianae*) more flame-cells on each side.

Cercaria Alariae-mustelae Bosma, from *Planorbula*, has two pairs of gland-cells, latero-posterior to the acetabulum; but the cercaria is so very much smaller, and differs in so many other respects from *C. amerianmae* that no close relationship seems possible. *Cercaria F* Harper, closely resembling the above, is also debarred from near relationship with *C. amerianmae*. The cercaria of *Strigea tarda* Steenstrup has four pre-acetabular gland-cells, but the excretory formula is $2[(1+1+1)+(2+(2))]=14$, and it is said to develop into a tetracotyle within the primary host, *Limnaea*.

Hence, it is clear that while the systematic position of *C. amerianmae* cannot be fully established, its affinities are with the Diplostomes. Some Diplostomes in which the fore and hind-body are scarcely differentiated externally have been assigned to *Tylodelphys* whose type is *T. clavatum* (Nordm.). The larva of the latter is also known as *T. rachidis.* Another species is *T. excavata* (Rud.), recorded from Ciconiidae and rarely from Ardeidae and Podicipididae, the metacercaria being *T. rachiaeum* which occurs in the vertebral canal of frogs, *Rana* spp., in Europe, while the cercaria occurs in *Planorbis.* In the cercaria of *T. excavata* the four gland-cells are arranged in front of the ventral sucker, as in *C. ameriannae.* In the later stages pseudosuckers are present but are weakly developed. The structures have not been recognized in our metacercaria. In another *Tylodelphys, T. clavata,* from *Ardea* and *Circus,* the metacercaria occurs in the vitreous humour of the eye of European freshwater fishes.

Summary.

Furcocercaria lessoni, a new species of Strigeid larva from *Limnaea* and *Planorbis,* from the River Murray, is a pharyngeal, distome, longifurcate cercaria, with four pairs of glands and two excretory commissures. The metacercaria is a Tetracotyle encysting in freshwater leeches, and the adult is probably a species of *Apatemon.*

C. ameriannae, a distome, longifurcate, pharyngeal cercaria from *Amerianna pectorosa,* is characterized by the possession of four pre-acetabular gland-cells and of an excretory system comprising 18 flame-cells and lacking commissures. The cercaria penetrates tadpoles and develops into an encysted metacercaria of the *Diplostomulum* type. The adult is probably Diplostome.

ADDENDUM.

Since this paper went to press, one by Olivier (*Tr. Amer. Micr. Soc.*, 61, 1942, 168–179) has come to our notice. In it is described *C. elodes* which resembles *C. ameriannae* more closely than does any other known to us, the only significant difference being that the four penetration gland-cells lie posterior to the ventral sucker in *C. elodes,* and the anterior to that organ in *C. ameriannae.* In both species the metacercaria develops in the notochord of tadpoles into a diplostomulum. It seems certain, therefore, that the two larvae are very closely related and may represent young stages of two different species of the same genus.

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